

**MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedures**

SOP #: MDNR-WQMS-015A EFFECTIVE DATE: April 21, 2005

SOP TITLE: Sample Collection and Field Handling Procedures for Chlorophyll *a*
Analysis of Phytoplankton

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SUMMARY OF REVISIONS: This SOP has been revised to expand the discussion on field
filtering and sample handling techniques. In addition, a
required equipment list has been added.

APPLICABILITY: The procedures outlined in this SOP apply to all ESP personnel
who collect surface water samples to be analyzed for
phytoplankton chlorophyll by the ESP laboratory.

DISTRIBUTION: MDNR Intranet
ESP, SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed				
Initials				

1.0 SCOPE AND APPLICABILITY

This Standard Operating Procedure provides Environmental Services Program (ESP) staff with guidance on collection and handling of surface water samples to be analyzed for phytoplankton that contain chlorophyll.

2.0 PERSONNEL QUALIFICATIONS

Field personnel must have a working knowledge of field sample collection procedures. Staff shall have, at a minimum, attended the department-sponsored Inspection and Enforcement training or received training from another MDNR employee knowledgeable on proper sample collection procedures.

3.0 HEALTH AND SAFETY

Field activities involving the collection of chlorophyll measurements may involve working in or around various waste streams. Field personnel should protect themselves by wearing the appropriate level of personal protective equipment such as disposable gloves and waders.

4.0 GENERAL OVERVIEW

Assessing biomass of the phytoplankton community in natural waters is helpful in determining the productivity or trophic condition of the water. One of the most direct and accurate measurements is to determine the concentration of photosynthetic pigments, especially chlorophyll, in water. Chlorophyll *a*, *b*, *c*, and several carotenoid pigments are all present in the phytoplankton community although chlorophyll *a* is the most abundant. Analysis of chlorophyll is often restricted to chlorophyll *a* alone.

Currently, ESP Water Quality Monitoring Section (WQMS) staff utilizes fluorometry to determine chlorophyll *a* concentrations in water (see MDNR-WQMS-110). The chlorophyll is measured after extracting it from cells using organic solvents. The extracted chlorophyll is expressed as a concentration of chlorophyll *a* per unit volume (e.g. ug/L).

5.0 SAMPLING CONSIDERATIONS

- 5.1 Specific sampling strategies will depend on the scope of the study, but in general, should conform to the principles outlined in MDNR-FSS-005.
- 5.2 Depending on the objectives of the study, samples may be collected from specific areas such as mid-lake/deep-water areas, riffles, shorelines, coves, etc.

- 5.3 In standing bodies of water, an adequate sampling program must account for the erratic distribution of phytoplankton. Multiple samples should be collected to obtain a representative estimate of chlorophyll concentrations. In addition, many waterbodies that are not well-mixed form vertical gradients of temperature, oxygen, nutrients and available sunlight. Each of these factors affects the distribution of phytoplankton throughout the water column. Thus, the sampler must consider whether depth integrated samples or discrete surface samples should be collected.
- 5.4 It should be noted that samples collected from water which is acidic should be filtered and processed as soon as possible. This helps minimize chlorophyll degradation caused by residual acidic water in the filter. Thus, it is essential for the sampler to determine the pH of water to be sampled using methods described in MDNR-FSS-100.

6.0 SAMPLING EQUIPMENT

The following equipment will be needed in the field during sample collection and filtering:

disposable pipettes	airtight/opaque container	cooler/ice
desiccant	nitrile gloves	paper clips
hand vacuum/pressure pump	filter funnel and flask	pencils
cubitainers	aluminum foil	trash bags
50-mL graduated cylinder	paper towels	forceps
DI water dispenser	Whatman paper pads (110-mm diameter)	
glass fiber filters (see note 1)	buffered deionized water (see note 2)	

Notes:

1. Glass fiber filters are type A/E, 47-mm diameter with 1- μ m pore size. WQMS typically use Pall AE P/N 61631, Millipore AP4004799, or equivalent brand.
2. Deionized water typically exhibits a pH between 5 and 6. DI water utilized for chlorophyll analyses should be buffered with MgCO_3 to pH 7 or greater. This can be accomplished by adding 10 mg of MgCO_3 to 1000 mL of DI water.

7.0 SAMPLE COLLECTION, CONTAINERS, PRESERVATIVES, AND STORAGE

- 7.1 Collect a grab sample of water in a cubitainer or other clean container (glass or plastic). A minimum volume of 100 mL should be obtained. Refer to MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations* for additional information.
- 7.2 Refer to MDNR-FSS-005 for the proper collection of grab samples and general sampling considerations. Each sample container should be labeled with a sample number. The sample number as well as the date and time of collection should be documented on the Chain-of-Custody Record (refer

to MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record* and MDNR-FSS-003 *Sample Numbering and Labeling*).

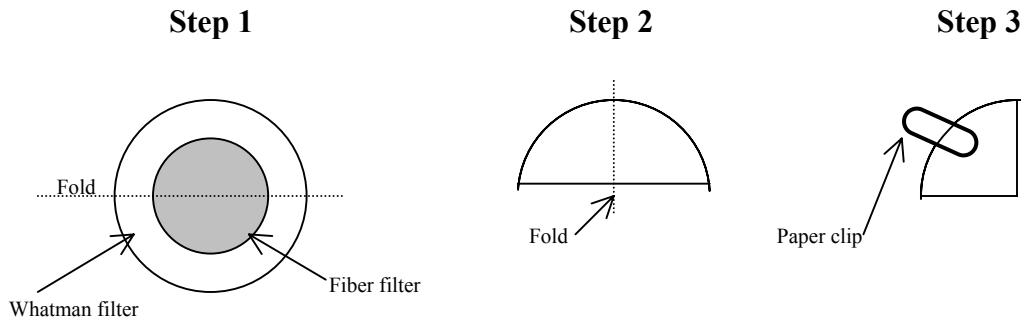
- 7.3 No chemical preservatives should be added to the water sample.
- 7.4 Samples that cannot be filtered immediately should be placed in a cooler with ice. The sample containers must be kept in the dark. The maximum holding time for unfiltered samples should not exceed six hours.

8.0 SAMPLE FILTERING

Water samples must be filtered as soon as possible after collection in order to separate phytoplankton from the water. In most cases this will be performed in the field. Once this task has been completed, the filter is retained as the sample and the water is discarded.

- 8.1 Filtering should be performed in an area of subdued light to avoid chlorophyll degradation. Filtering may be performed outside but direct sunlight must be avoided.
- 8.2 Skin secretions, such as oils and perspiration, breakdown chlorophyll *a*. Thus, nitrile gloves should be worn when handling samples.
- 8.3 Prior to filtering, gently agitate the water sample and measure out an appropriate volume using a graduated cylinder. Typically, WQMS staff filter 10 to 50 mL of water per sample. The amount of water to be filtered varies depending on turbidity and the presence of algae. For instance, 50 mL of water would typically be filtered from a sample collected in a clear Ozark stream. Conversely, only 10 mL of filtered water would be required when sampling lagoon effluent that is turbid due to algae.
- 8.4 Using a hand vacuum pump, filter funnel, and flask, pass the known volume of sample through a 47-mm glass type AE fiber filter (1 μ m pore size). Rinse the sides of the filter funnel with buffered deionized water, then pass the rinse water through the filter as well. Note that the amount of vacuum pressure applied should not exceed 10 psi since filtering at higher pressures can rupture the algae cells.
- 8.5 Using forceps, remove the glass fiber filter from the funnel and place it on a paper towel (chlorophyll side up). Fold the glass fiber filter in half with chlorophyll side folding upon itself, then utilize the paper towel to blot excess moisture from the filter. Place the dried fiber filter on a Whatman pad. Fold the Whatman pad (with the glass fiber filter inside) in half, then fold in half again. A paper clip can be utilized to keep the pad from unfolding (see diagram below). The sample number and volume of water filtered should be written on the outside of the Whatman pad using a

pencil (do not use a pen or magic marker). If necessary, the assigned sample label can also be placed under the paper clip.



- 8.6 Two glass fiber filters should be prepared for each water sample and stored in separate Whatman pads.
- 8.7 Place the folded filters in an airtight, opaque container that has been filled with desiccant. (Note: Aluminum foil can be wrapped around a container to ensure that the filters are not exposed to any light source.) The container should immediately be placed in a cooler with ice until delivery to the ESP laboratory.
- 8.8 The filter funnel apparatus and graduated cylinder must be decontaminated between samples by triple rinsing with buffered distilled water.
- 8.9 Filtered sample water should be discarded in the field if possible. Typically, this can be accomplished by simply pouring the water on the ground. In some instances, it may be necessary to place the filtered water in a sanitary sewer system.

9.0 FILTER HANDLING AND STORAGE

- 9.1 Typically, WQMS staff are responsible for analysis of chlorophyll. ESP - Chemical Analysis Section staff does not perform this analysis.
- 9.2 In most cases, samples will be transported to the ESP laboratory for analysis. WQMS staff should check the desiccant and replace if spent. The desiccant turns from a bright blue to light blue or white when spent.

- 9.3 Chlorophyll sample filters should be logged onto a chain-of-custody document, transported to the ESP laboratory, and submitted to Sample Receiving as described in MDNR-FSS-018. The term “Chlorophyll – WQMS – Phytoplankton” should be specified in the ANALYSES block of the chain-of-custody document. (Note: Each filter pad is counted as one container.)
- 9.4 Once the chlorophyll filters are accepted by ESP-Sample Receiving, store the entire container (desiccant and filters) in a freezer until analysis.
- 9.5 The filters can be stored in a freezer up to one month, but it is best to perform the extraction and analysis quickly. Samples collected from water that is known to be acidic should be processed as soon as possible. This prevents chlorophyll degradation caused by residual acidic water in the filter. Please refer to MDNR-WQMS-207 for this procedure.

10.0 REFERENCES

MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations.*

MDNR-FSS-002 *Field Sheet and Chain of Custody Record*

MDNR-FSS-003 *Sample Numbering and Labeling*

MDNR-FSS-004 *Field Documentation*

MDNR-FSS-005 *General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows.*

MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab.*

MDNR-FSS-100 *Field Analysis of Water Samples for pH.*

MDNR-WQMS-207 *Laboratory Extraction Procedure for Chlorophyll Analysis by Fluorometric Techniques.*

Standard Methods for the Examination of Water and Wastewater, 1998, 20th Edition.

Limnology, second edition, 1983, Wetzel, Robert G.